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Post-insemination milk progesterone concentration and embryo survival in dairy cows

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Abstract

Logistic regression analysis was used to evaluate the relationship between post-insemination milk progesterone concentration and embryo survival, and between milk yield and milk progesterone concentration. Milk samples were collected on Days 1, 4, 5, 6, and 7 (insemination = Day 0) following 871 inseminations in spring-calving dairy cows. Milk progesterone concentrations were measured by enzyme-immunoassay and pregnancy diagnosis was conducted with transrectal ultrasonography at approximately Day 30. There was a negative linear relationship ($P < 0.01$) between milk progesterone concentration on Day 4 and embryo survival while, in contrast, there was a positive linear and quadratic relationship between milk progesterone concentration on Days 5, 6 and 7 ($P < 0.05$) and also between the rate of change in progesterone concentrations between Days 4 and 7 inclusive and embryo survival ($P < 0.05$). There was a weak negative linear relationship between average daily milk yield at the time of insemination and milk progesterone concentrations ($P < 0.001$). There was no association between many production parameters, including liveweight and body condition score measured at various stages between calving and insemination, and milk progesterone concentration between Days 4 and 7 inclusive ($P > 0.05$). In conclusion, low progesterone during Days 5–7 (after insemination) was associated with low fertility in dairy cows

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and there were indications of a range of progesterone concentrations within which embryo survival was maximal.

Keywords: Milk progesterone; Embryo survival; Milk production; Body condition score; Dairy cow

1. Introduction

The timing and rate of the post-ovulatory increase in systemic concentrations of progesterone as well as the absolute concentration may be important in determining subsequent embryo survival in cattle. Low systemic progesterone concentrations on Day 5 post-ovulation [1–3] or a delay in the normal rise in progesterone between Days 4 and 5 post-ovulation [3–6] have been associated with reduced pregnancy rates, although the numbers of animals employed in some of these studies have been limited. Furthermore, a delay in the normal rise in progesterone in the early luteal phase resulted in smaller embryos with sub-optimal interferon tau secretion and perhaps a reduced ability to inhibit luteolysis [7–9]. However, while some studies have shown a positive association between progesterone and embryo survival rate in cattle [10], others have not recorded any such association [11–13]. The inconsistency between these reports may be due, in part, to variations in the number of cattle, and in part to the statistical analysis procedures used. In many of the studies, cows were first divided into pregnant or non-pregnant groups and mean concentrations of progesterone were then compared between the groups. This simplistic approach fails to take into account the dynamics of the relationship between progesterone concentration and embryo survival.

The objective of this study was to use logistic regression analysis to establish, in spring-calving dairy cows, the relationships between: (i) milk progesterone concentrations on Days 4, 5, 6, and 7 post-insemination and early embryo survival; (ii) the rate of change in milk progesterone concentrations from Days 4–7 inclusive and early embryo survival; and (iii) cow production measurements and milk progesterone concentrations on Days 4–7 inclusive.

2. Materials and methods

2.1. Animals

This study was conducted over a 5-mo period from April to August and involved six spring-calving dairy herds with a total of 523 cows. Average herd size was 87 (range: 44–161), with an average annual replacement rate of 25%. Five of the herds comprised Holstein Friesian cows only ($n = 362$) while the sixth was a mixed breed herd comprised of Holstein Friesian ($n = 46$), Montbeliarde ($n = 28$), Montbeliarde × Holstein Friesian ($n = 26$), Normande ($n = 12$), Normande × Holstein Friesian ($n = 23$) and Norwegian Red ($n = 26$) cows. Parity ranged from 1 to 10 and average annual milk yield (across all herds) was 6043 ± 1220 kg (mean ± S.D.).

Throughout the duration of the trial, individual cow records including cow identification number, insemination sire, dates of observed estrus, and breeding dates were recorded.

Across all herds, cows were fed grass silage during the winter period, with a daily supplement of 2–4 kg of concentrates. The mean calving date for the 6 herds was the February 27 and the mean turn out date to pasture was March 11. Cows were fed 2–7 kg of concentrates at pasture each day. The mean dates for the start and finish of breeding were April 6 and August 6, respectively.

2.2. Milk sampling

Milk samples, representative of the entire milking, were collected during the morning milking on Days 1, 4, 5, 6, and 7 (Day 0 = day of insemination; n = 871 inseminations). Samples were collected in 30 mL universal plastic containers containing a Lactab Mark III preservative tablet (Thompson and Capper Ltd., Cheshire, England). Following collection, samples were stored at 4 °C until assayed for progesterone.

2.3. Progesterone enzyme immunoassay

Whole-milk progesterone concentration was measured by enzyme immunoassay [14], using a commercial EIA (Ridgeway Science Ltd., Gloucester, UK). The intra- and inter-assay coefficients of variation for low, medium and high control samples were 5.7, 6.2 and 3.5%, and 17.0, 8.4 and 8.2%, respectively. The minimum detectable concentration of the assay was 1.0 ng/mL.

2.4. Estrus detection and insemination

Estrus detection was routinely conducted from 2 week after calving to the end of the breeding season. In each herd, cows were observed at the morning milking, noon, evening milking, and at 22:00 h daily. Each observation period lasted at least 30 min. Tail paint [15] was used routinely in each herd to help detect estrus. On all farms, cows were inseminated during standing estrus by experienced inseminators. Cows first detected in estrus at the morning milking were inseminated that morning while those detected at noon, the evening milking or at 22:00 h were inseminated the following morning. Semen from sires of known normal to high fertility was used. One inseminator was used for each herd and the insemination protocol did not differ between successive inseminations.

2.5. Pregnancy diagnosis

Pregnancy diagnosis was carried by an experienced operator on Day 30 post-insemination by transrectal ultrasonography, with an ALOKA SSD 500 V scanner with 5 MHz transducer (Aloka Ltd., Tokyo, Japan).

2.6. Milk yield and composition

Milk yield was recorded weekly for each cow, starting 5 days after parturition. The milk fat, protein and lactose concentrations were determined using a Milkoscan 203 (Foss Electric, Denmark), from successive morning and evening samples collected once weekly.

Average daily milk energy output (EV_1) [16] was recorded up to 63 days post-partum, along with average daily yields of milk, fat, protein and lactose that were estimated based on cumulative milk production from 5 to 63 days post-partum. Average daily milk yield in a 21 days period around each insemination was calculated.

2.7. Liveweight measurement and body condition scoring

Liveweight (LW) and body condition score (BCS) were recorded once every 2 week from parturition to approximately 60–80 days after the end of the breeding season. Body condition scores were allocated on a scale of 0–5 (in increments of 0.25), with a score of 0 representing extremely thin or emaciated cows and 5 representing extremely fat or obese cows [17]. Liveweight and body condition score were also recorded at the time of calving (CALVLW and CALVBCS, respectively) and 63 days after calving (LW60 and BCS60, respectively).

2.8. Statistical analysis

Logistic regression analysis [18] was used to evaluate the relationship between milk progesterone concentration on Days 4, 5, 6, and 7 and also the change in milk progesterone concentration between Days 4 and 7 inclusive, and subsequent embryo survival (Proc LOGISTIC [19]), with progesterone concentrations fitted both as linear and quadratic terms. This first derivative of this relationship was then used to determine the concentration of milk progesterone associated with maximal embryo survival. Cows with >3 ng/mL of progesterone on Day 1 were deemed to have commenced luteal activity [20] and were excluded from the analysis. Additionally, cows with no change in milk progesterone concentration from Days 1 to 7 were deemed anovulatory and excluded from the analysis. Backwards, stepwise multiple regression analysis was used to evaluate the relationships between: average daily milk energy output (EV_1) to 63 days post-partum; average daily yield of milk, fat and protein (based on cumulative milk production to 63 days post-partum); average daily milk yield at insemination; parity number; calving date; liveweight at calving; liveweight 63 days post-partum; change in liveweight between calving and 63 days post-calving; change in liveweight between calving and insemination; body condition score at calving; body condition score 63 days post-partum; change in body condition score between calving and 63 days post-calving, or change in body condition score between calving and insemination; and milk progesterone concentration on Days 4, 5, 6, and 7 post-insemination (Proc REG [19]).

3. Results

3.1. Relationship between insemination number and percentage pregnant

The relationship between insemination number and percentage of cows pregnant is shown in Table 1. There was an insemination number x herd interaction ($P < 0.05$), with no overall difference between herds or insemination number ($P > 0.05$).

Table 1
Effects of service number on pregnancy rate in six herds of dairy cows

Service no.	Herd A	Herd B	Herd C	Herd D	Herd E	Herd F	Total
First	60/161, 37%	26/45, 58%	25/44, 56%	21/45, 47%	78/150, 52%	40/78, 51%	250/523, 48%
Second	45/93, 48%	11/17, 65%	7/12, 58%	8/21, 38%	32/69, 28%	17/34, 50%	120/246, 44%
Third	18/30, 60%	4/5, 80%	2/3, 67%	2/8, 25%	14/40, 35%	8/16, 50%	48/102, 47%
Total	123/161, 76%	41/45, 91%	34/44, 77%	31/45, 68%	124/150, 82%	65/78, 83%	418/523, 47%

3.2. Relationship between milk progesterone concentration on Days 4–7 post-insemination and embryo survival

The relationship between milk progesterone on Days 4–7 and embryo survival is presented in Table 2 and in Fig. 1a–e, inclusive. The proportion of cows pregnant/cows inseminated is also shown for each progesterone concentration range. The odds ratios are presented (Table 2), with corresponding 95% confidence intervals, significance values, optimal concentration of progesterone for maximal embryo survival, and the percentage of cows with progesterone concentrations below this optimum. Although there was no herd × progesterone interaction ($P > 0.05$), there was an effect of herd on progesterone, with one herd (Herd C) having lower progesterone concentrations than the other herds ($P < 0.05$) on Days 4–7.

There was a negative linear relationship ($P < 0.01$) between the concentration of milk progesterone on Day 4 and subsequent embryo survival (Fig. 1a). There was a positive linear and quadratic relationship ($P < 0.05$) between the concentration of milk progesterone on Day 5 and subsequent embryo survival (Fig. 1b) with an optimum milk progesterone concentration of 7.4 ng/mL (Table 2). Sixty percent of animals had concentrations lower than the optimum and the remaining 40% had concentrations greater than the optimum.

There was a positive linear and quadratic relationship ($P < 0.05$) between the concentration of milk progesterone on Day 6 and subsequent embryo survival (Fig. 1c). Based on the logistic regression curve, the optimum milk progesterone concentration consistent with maximum embryo survival was 13.2 ng/mL (80 and 20%, respectively, had smaller and larger slopes).

There was a positive linear and quadratic relationship ($P < 0.05$) between the concentration of milk progesterone on Day 7 and subsequent embryo survival (Fig. 1d). Based on the logistic regression curve, the optimum milk progesterone concentration consistent with maximum embryo survival was 16.8 ng/mL (75 and 25% of cows, respectively, had smaller and larger slopes).

3.3. Relationship between the rate of change in milk progesterone concentration from Days 4–7 and embryo survival

There was a positive linear and quadratic relationship ($P < 0.05$) between the rate of change in milk progesterone concentration from Days 4–7 inclusive and subsequent embryo survival (Fig. 1e). Based on the logistic regression curve, the optimum rate of increase in milk progesterone concentration (slope) on Days 4–7 consistent with maximum

Table 2
Relationships between milk progesterone concentration on Days 4–7 inclusive post-insemination, rate of change in milk progesterone from Days 4–7 inclusive, and embryo survival following logistic regression and the associated odds ratios, 95% confidence limits, level of significance, concentration of progesterone optimal for embryo survival and the percentage of cows below the optimal

Measurement	Effect	Odds ratio	95% confidence interval	Significance	Optimal progesterone concentration (ng/mL)	Percentage of cows below optimal progesterone concentration
Day 4 progesterone concentration	Linear	−0.091	0.849–0.975	$P < 0.01$	N/A	N/A
Day 5 progesterone concentration	Linear	1.200	1.013–1.422	$P < 0.05$	7.4	60
	Quadratic	0.988	0.978–0.998	$P < 0.05$		
Day 6 progesterone concentration	Linear	1.148	1.017–1.296	$P < 0.05$	13.2	80
	Quadratic	0.995	0.990–1.000	$P < 0.05$		
Day 7 progesterone concentration	Linear	1.111	1.005–1.227	$P < 0.05$	16.8	75
	Quadratic	0.997	0.994–1.000	$P < 0.05$		
Rate of change in progesterone concentration from Days 4–7	Linear	1.514	1.125–2.038	$P < 0.01$	4.7	85
	Quadratic	0.957	0.922–0.993	$P < 0.05$		

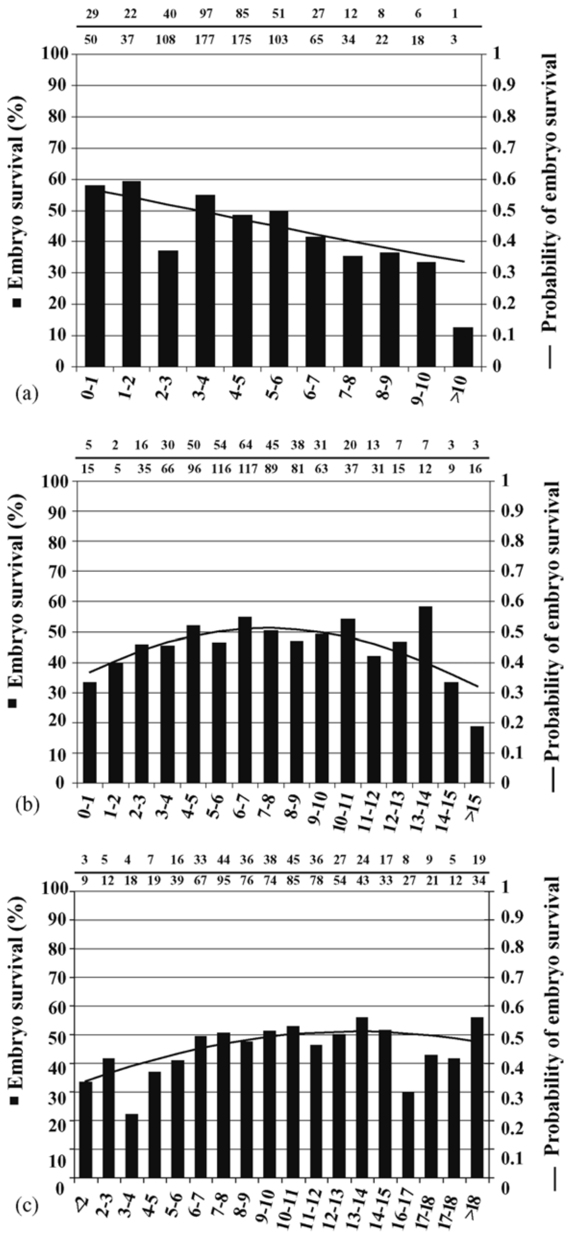

Fig. 1. Percentage embryo survival and logistic regression curve of the relationship between the concentration of milk progesterone on Day 4 (a), Day 5 (b), Day 6 (c) Day 7 (d) and the rate of change in milk progesterone concentration from Days 4–7 post-insemination and the probability of embryo survival (e). The proportion pregnant/inseminated is also indicated for each category of progesterone.

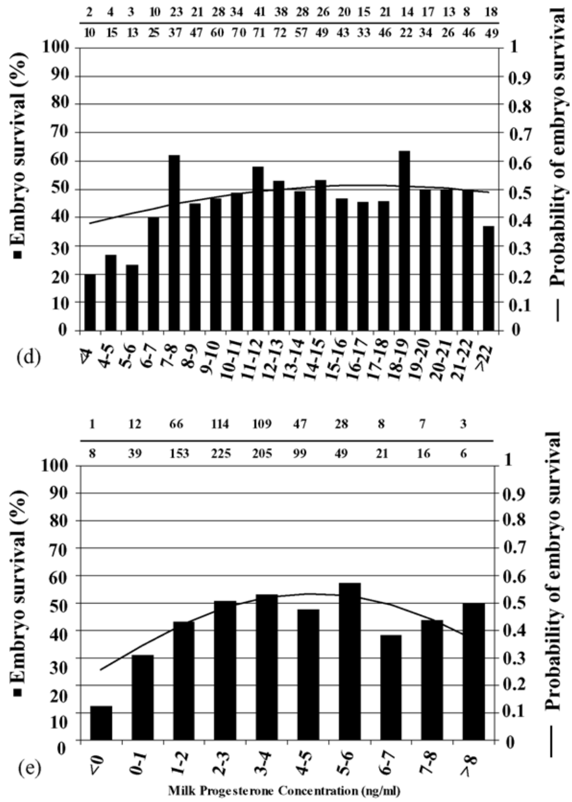

Fig. 1. (*Continued*).

embryo survival was 4.7 ng/mL/day (85 and 15% of cows, respectively, had smaller and larger slopes).

3.4. Relationship between cow production measurements and milk progesterone concentration

Data relating to cow weights, body condition score and production parameter from calving to 63 days post-calving are shown in Table 3. There was no difference between herds for any of the production parameters measured ($P > 0.05$). Milk progesterone concentrations on Days 4–7 were not associated ($P > 0.05$) with any of the independent variables, including average daily milk energy output (EV_1) to 63 days post-partum, fat and protein based on cumulative milk production to 63 days, parity number, calving date, liveweight at calving, liveweight at 63 days, change in liveweight between calving and 63 days, change in liveweight between calving and insemination, body condition score at calving, body condition score at 63 days, change in body condition score between calving and 63 days, or change in body condition score between calving and insemination. For each

Table 3
Cow body weight (wt.), body condition score (BCS), milk yield, and percentage milk fat, protein and lactose from calving to 63 days post-calving in six dairy herds (– = no data available)

	Herd A	Herd B	Herd C	Herd D	Herd E	Herd F
No. of cows	161	45	44	45	150	78
Wt. at calving (kg)	535 ± 3.8	585 ± 8.3	–	583 ± 8.3	577 ± 4	538 ± 6.0
BCS at calving	3.0 ± 0.04	3.3 ± 0.05	3.0 ± 0.04	3.08 ± 0.05	3.1 ± 0.02	3.0 ± 0.02
Wt. 63 days post-calving	516 ± 3.4	578 ± 8.5	NA	576 ± 9.4	575 ± 3.9	535 ± 5.8
BCS 63 days post-calving	2.9 ± 0.02	3.2 ± 0.05	3.0 ± 0.05	2.7 ± 0.05	2.8 ± 0.02	2.9 ± 0.02
Milk yield to 63 days (kg)	24.0 ± 0.29	30.2 ± 0.63	–	–	30.9 ± 0.30	27.4 ± 0.45
Fat to 63 days (%)	3.8 ± 0.02	4.0 ± 0.04	–	–	4.16 ± 0.02	4.4 ± 0.30
Protein 63 days (%)	3.2 ± 0.01	3.2 ± 0.02	–	–	3.3 ± 0.01	3.4 ± 0.02
Lactose to 63 days (%)	4.8 ± 0.01	4.8 ± 0.02	–	–	4.7 ± 0.01	4.8 ± 0.01

Table 4
Relationships between milk progesterone concentrations on Days 4, 5, 6, and 7 post-insemination and average daily milk yield peri-insemination in dairy cows

Dependent variable	Independent variable
Day 4 Progesterone	$=-0.11(\text{ADMYPI}) + 7.11$; $P < 0.001$, $R^2 = 0.10$
Day 5 Progesterone	$=-0.18(\text{ADMYPI}) + 11.89$; $P < 0.001$, $R^2 = 0.13$
Day 6 Progesterone	$=-0.26(\text{ADMYPI}) + 17.21$; $P < 0.001$, $R^2 = 0.13$
Day 7 Progesterone	$=-0.35(\text{ADMYPI}) + 23.14$; $P < 0.001$, $R^2 = 0.15$

ADMYPI: aveage daily milk yield (kg) peri-insemination.

of Days 4, 5, 6, and 7, there was a negative linear relationship ($P < 0.001$) between progesterone concentration and average daily milk yield at the time of insemination (Table 4).

4. Discussion

In the present study, there was a strong positive relationship between milk progesterone concentrations on Days 5, 6 and 7 and the probability of subsequent embryo survival. There was no herd $\times$ progesterone interaction ($P > 0.05$), indicating that the relationships were consistent across herds. Although there was an effect of herd on progesterone concentration, with one herd (Herd C) having lower progesterone than the other herds on Days 4–7, herd was not retained in the model; in the context of the analysis, and in the absence of a significant interaction, it was not considered appropriate. In contrast, the relationship between progesterone concentration and fertility, regardless of overall progesterone concentration within the herd, was of principal interest. Although Herd C had the lowest progesterone concentration on all days, there was no significant effect of herd on overall fertility.

There was a linear and quadratic relationship between progesterone and embryo survival rate on Day 5, consistent with the data of Starbuck et al. [5,6]. Within the parameters of the current study, a milk progesterone concentration of 7.4 ng/mL on Day 5 was consistent with maximal embryo survival; lower or higher concentrations were associated with reduced embryo survival. Similarly, Starbuck et al. [5,6] reported that progesterone concentrations between 7 and 8 ng/mL on Day 5 were associated with maximal pregnancy rates, whereas concentrations that were lower or higher were associated with reduced fertility. Similar trends in the relationship between progesterone and embryo survival were observed on Days 6 and 7 in the current study, with maximal survival at concentrations of 13.2 and 16.8 ng/mL, respectively, on these two days. Ahmad et al. [10] found a significant difference between pregnant and non-pregnant cows in systemic progesterone concentrations 6–7 days after AI. In contrast, Bulman and Lamming [12] failed to find any association between embryo survival and either progesterone concentration on any day between Days 0 and 13 post-insemination, or the rate of increase in progesterone concentrations between Days 3 and 12. However, in that study, cows were classified as pregnant and non-pregnant prior to statistical analysis. Similarly, Lamming et al. [21] in a retrospective analysis based on thrice-weekly milk sampling, compared pregnant versus non-pregnant cows and found no evidence of lower progesterone concentrations on Days 0–6 post-insemination in cows that failed to conceive. However, the authors noted that there was a considerable range in progesterone concentrations in any cows on any given day, and that individual cows with low concentrations of progesterone may have been less likely to conceive. Furthermore, they reported that animals suffering early embryo loss had significantly lower concentrations of progesterone between Days 7 and 16 post-insemination than animals that maintained pregnancy.

Also, in contrast to the study of Bulman and Lamming [12], the results of the present study show a significant linear and quadratic relationship between the rate of change in the concentration of milk progesterone from Days 4–7 inclusive and subsequent embryo survival. Embryo survival rate was maximised when the rate of increase in the concentration of progesterone from Days 4–7 was 4.7 ng/mL/day. Similarly, Larson et al. [3], Darwash and Lamming [4] and Starbuck et al. [5,6] reported that a delay in the luteal phase rise in the concentration of progesterone after insemination was associated with decreased pregnancy rates in dairy cows.

In contrast to Days 5, 6 and 7, there was no clear relationship between milk progesterone concentration on Day 4 and embryo survival in the present study. Unfortunately, there are apparently no similar published studies with Day-4 progesterone data. It appears that progesterone concentration on Day 4 was negatively associated with embryo survival and that the highest embryo survival rate was associated with the lowest progesterone concentrations found. It was noteworthy that this does not exclude that there is an optimal concentration of progesterone on Day 4 that is below the detection limit of the assay. Consequently, identification of a possible linear and quadratic relationship between progesterone concentration and embryo survival (similar to that found for Days 5, 6 and 7), was not possible. That high progesterone concentrations on Day 4 were associated with low embryo survival rates could be attributed to excess progesterone creating an advanced or asynchronous uterine environment, with a detrimental effect on the very early embryo [22,23]. In that regard, temporal synchrony between the embryo and uterus is critical for optimal embryo survival both in cattle [24–26] and in sheep [22,27,28] and this synchrony is affected by systemic progesterone concentrations.

On Days 5, 6 and 7, the majority (60, 80 and 75%, respectively) of cows had concentrations of progesterone below the optimum, indicating that inadequate rather than excess progesterone is a greater problem on these days. That high progesterone concentrations on Days 4–7 were also detrimental to embryo survival was consistent with the observation of Starbuck et al. [5,6]; they showed that progesterone supplementation was effective in increasing pregnancy rate only in cows with low (1–2 ng/mL) milk progesterone concentrations. Supplementation of cows that had higher concentrations of progesterone on Day 5 had no beneficial effect on pregnancy rate. Based on the results of this study and that of Starbuck et al. [5,6], showing that both low and high concentrations of progesterone were both associated with a low embryo survival rate, it is not surprising that many of the earlier studies failed to detect significant differences in progesterone concentrations between pregnant and non-pregnant cows. Clearly if progesterone supplementation was to be effectively used to increase embryo survival, it would have to be in a targeted fashion rather than blanket treatment of all cows [29].

In an attempt to identify factors that predispose cows to having sub-optimal progesterone concentrations, the relationship between a number of production measurements including, milk yield, milk energy output, milk fat and milk protein yield and the systemic progesterone concentration was also examined. The only significant relationship detected was a negative linear relationship between average daily milk yield (around the time of insemination) and milk progesterone concentration on Days 4, 5, 6, and 7. This was in agreement with the report of Hommeida et al. [30] who found a negative relationship between the concentration of milk progesterone and milk yield during the first 15 days after insemination in Holstein Friesian cows. In the current study, the negative relationship may be a consequence of increased hepatic clearance rate of progesterone as a result of the greater hepatic metabolic activity required for milk synthesis [31,32], with a concurrent increase in steroid metabolism [33–35]. The negative relationships may also simply reflect a dilution effect of high milk yield. Other possible reasons for differences in systemic progesterone concentrations between cows include pre-ovulatory follicle diameter (which affects the number of granulosa cells and ultimately CL size and secretory activity), the number of ovulations (and corpora lutea), breed effects, post-partum interval, and negative energy balance [36–38].

In conclusion, both sub- and supra-optimal concentrations of progesterone from Days 4–7 after AI or a sub-optimal rate of increase in the concentration of progesterone during this interval were negatively associated with embryo survival rate. Although excessive progesterone concentrations were associated with reduced embryo survival, progesterone insufficiency appeared to be the most common problem.

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